

# Evidence That the Histone Methyltransferase Dot1 Mediates Global Genomic Repair by Methylating Histone H3 on Lysine 79\*

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Global genomic repair (GGR) and transcription coupled repair (TCR) are two pathways of nucleotide excision repair (NER) that differ in the damage recognition step. How NER factors, especially GGR factors, access DNA damage in the chromatin of eukaryotic cells has been poorly understood. Dot1, a histone methyltransferase required for methylation of histone H3 lysine 79 (H3K79), has been shown to confer yeast cells with resistance to DNA-damaging agents and play a role in activation of DNA damage checkpoints. Here, we show that Dot1 and H3K79 methylation are required for GGR in both nucleosomal core regions and internucleosomal linker DNA, but play no role in TCR. H3K79 trimethylation contributes to but is not absolutely required for GGR, and lower levels of H3K79 methylation (mono- and dimethylation) also promote GGR. Our results also indicate that the roles of Dot1 and H3K79 methylation in GGR are not achieved by either activating DNA damage checkpoints or regulating the expression of the GGR-specific factor Rad16. Rather, the methylated H3K79 may serve as a docking site for the GGR machinery on the chromatin. Our studies identified a novel GGR-specific NER factor and unveiled the critical link between a covalent histone modification and GGR.

Nucleotide excision repair (NER)<sup>2</sup> is a highly conserved DNA repair mechanism that removes a wide variety of bulky, helix-distorting lesions that generally obstruct transcription and normal replication, such as UV-induced cyclobutane pyrimidine dimers (CPDs) (1). Transcription-coupled repair (TCR) is a specialized NER pathway that is dedicated to rapid repair in the transcribed strand (TS) of active genes and is believed to be initiated by an RNA polymerase stalled at a lesion in the TS (2). The genome-wide NER, which includes repair in the nontranscribed strand (NTS) of actively transcribed genes, is termed global genomic repair (GGR) to be distinguished from TCR. The two NER pathways share most of the common repair factors and differ only in the damage recognition step (1).

In the budding yeast *Saccharomyces cerevisiae*, Rad7, Rad16, and Elc1 are specifically required for GGR (3, 4). The exact roles

of these proteins in GGR are not yet clear. The Rad7-Rad16 complex may act as an ATP-dependent motor that translocates along the DNA in search of damage, and upon encountering a lesion, the complex is stalled, which may remodel and open damaged chromatin, thereby facilitating recruitment of other NER factors (5). Elc1 has been shown to be a component of a ubiquitin ligase that contains Rad7 and Rad16 (6). It has also been suggested that Elc1 is a component of another ubiquitin ligase complex, which contains Ela1, Cul3, and Roc1 but not Rad7 and Rad16 (7). The role of Elc1 in GGR does not seem to be subsidiary to that of Rad7 and Rad16 (3).

The molecular basis of chromatin dynamics during NER in eukaryotic cells is still not well understood (8, 9). The basic repeating component of chromatin is the nucleosome, which comprises 146 bp of DNA wrapped around an octamer of the four core histone proteins H2A, H2B, H3, and H4 (10). Histones are subject to a multitude of post-translational modifications, including acetylation, methylation, phosphorylation, sumoylation, and ubiquitination (11). Some of these modifications may modulate the NER process (8, 9). However, the effects of histone modifications on NER in living cells documented so far are generally quite modest and are most likely due to the alteration of chromatin compaction and/or stability. It has been unknown whether the NER, especially GGR, machinery relies on a specific histone modification to gain access to a lesion in the chromatin.

Dot1 is a histone methyltransferase required for methylation of histone H3 lysine 79 (H3K79) (12). *dot1* mutants are sensitive to UV light (13) and have a defect in activation of DNA damage checkpoints (14). In this paper, we present evidence that Dot1, by methylating H3K79, plays a pivotal role in GGR but is entirely dispensable for TCR. Our studies identified a novel GGR-specific NER factor and unveiled the critical link between a covalent histone modification and GGR.

## EXPERIMENTAL PROCEDURES

**Yeast Strains and Plasmids**—Wild-type yeast strains used were Y452 (*MAT $\alpha$  ura3-52 his3-1 leu2-3 leu2-112*) and BY4741 (*MAT $\alpha$  his3 $\Delta$ 1 leu2 $\Delta$ 0 met15 $\Delta$ 0 ura3 $\Delta$ 0*). *bre1 $\Delta$*  cells (*MAT $\alpha$  his3 $\Delta$ 1 leu2 $\Delta$ 0 met15 $\Delta$ 0 ura3 $\Delta$ 0 bre1::Kan*) were from Open Biosystems. Cells expressing mutant H3K79A and H2BK123A and their isogenic wild-type strains YBL574 and FY406 (15) were kindly provided by Dr. Ali Shilatifard (Stowers Institute for Medical Research). *DOT1* and *RAD16* deletions were created using procedures described previously (16).

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<sup>2</sup>The abbreviations used are: NER, nucleotide excision repair; CPD, cyclobutane pyrimidine dimer; GGR, global genomic repair; H2BK123, histone H2B lysine 123; H3K79, histone H3 lysine 79; NTS, nontranscribed strand; TCR, transcription-coupled repair; TS, transcribed strand.

pGAL-RAD16, a plasmid encoding 3×myc-tagged Rad16 under the control of the *GAL1* promoter, was created using vector pESC-URA (Stratagene). Two consecutive myc tag sequences were inserted in-frame downstream of the native single myc sequence of the vector. The *RAD16* gene coding sequence was inserted in-frame upstream of the 3×myc sequences. The plasmid can complement the deletion of the genomic *RAD16* gene for GGR, indicating that the plasmid-encoded Rad16 is functional.

**UV Irradiation, Repair Incubation, and Genomic DNA Isolation**—Yeast cells were grown at 30 °C in minimal medium containing 2% glucose or 2% galactose (to induce a gene under the control of the *GAL1* promoter) to late log phase ( $A_{600} \approx 1.0$ ), washed twice with ice-cold water, resuspended in ice-cold 2% glucose (for glucose cultures) or 2% galactose (for galactose cultures), and irradiated with 80 J/m<sup>2</sup> of 254-nm UV light. One-tenth volume of a stock solution containing 10% yeast extract and 20% peptone was added to the irradiated cell suspension. The cells were incubated at 30 °C in the dark to allow them to repair their DNA, and aliquots were collected at different time points. Genomic DNA was isolated from the cells as described previously (16).

**NER Analysis of UV-induced CPDs at Nucleotide Resolution**—To measure the induction and repair of CPDs at individual sites in each strand of the *RPB2* gene, we used the method that allows for strand-specific “fishing out” and labeling of a DNA fragment of interest (17–19). Briefly, ~1 μg of each of the total genomic DNA samples was digested with *DraI* to release the *RPB2* gene fragment of 1144 bp, which bears the 197-bp sequence upstream and the 947-bp sequence downstream of the transcription start site of the gene. The CPDs induced (in samples of 0-h repair) or remaining (in samples of different times of repair incubation) were converted to single-strand breaks by treatment with an excess amount of T4 endonuclease V, which specifically cleaves the DNA at CPD sites (20). Two biotinylated oligonucleotides were then used to specifically fish out and label the TS and NTS of the *RPB2* gene fragment, respectively. One pmol of one of the oligonucleotides was mixed with each of the samples. The mixtures were heated at 95 °C for 5 min to denature the DNA and then cooled to an annealing temperature of approximately 50 °C, to hybridize one strand of the *RPB2* fragment to the respective biotinylated oligonucleotide. One hundred μg of streptavidin magnetic beads (Dynabeads M-280 Streptavidin; Invitrogen) was added to each of the mixtures to capture the strand of the *RPB2* fragment hybridized to the biotinylated oligonucleotide. The other unwanted genomic DNA fragments were washed away by incubating the beads in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at the annealing temperature (50 °C). The fragments captured on the magnetic beads were 3′ end-labeled with [ $\alpha$ -<sup>32</sup>P]dATP and Sequenase Version 2 (US Biochemicals). The labeled fragments were eluted from the magnetic beads with a DNA sequencing gel loading buffer at 50 °C and resolved on sequencing gels. The gels were dried and exposed to a PhosphorImager screen (Bio-Rad). The signal intensities at gel bands corresponding to CPD sites were quantified by using Quantity One software (Bio-Rad). The percent CPDs remaining at individual sites after different times of repair incubation were calculated, and the times

required for repairing 50% of CPDs ( $t_{1/2}$ ) were obtained by either linear or second-order polynomial regression.

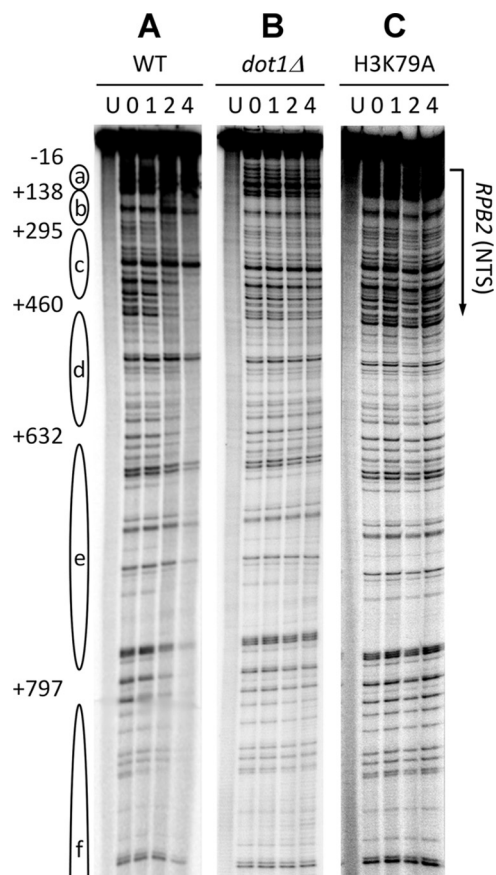
## RESULTS

***Dot1 Is Required for GGR***—It has been established that NER rates in the NTS of an active gene reflect GGR (1). In theory, NER in either strand of an absolutely repressed gene may also reflect GGR. However, “noise” transcription commonly occurs in both strands of supposedly repressed genes in eukaryotic cells (21). The noise transcription cannot be detected by traditional ways but may be able to initiate a certain level of TCR, which can be confused with GGR (22). Active transcription from the TS of a gene may prevent noise transcription from the NTS. Therefore, NER in the NTS of an actively transcribed gene may reflect GGR better than that in either strand of a repressed gene. Our previous studies have shown that NER in the NTS of the constitutively transcribed *RPB2* gene is absolutely dependent on the GGR-specific factors Rad7, Rad16, and Elc1, and therefore appears to exclusively reflect GGR (3, 22).

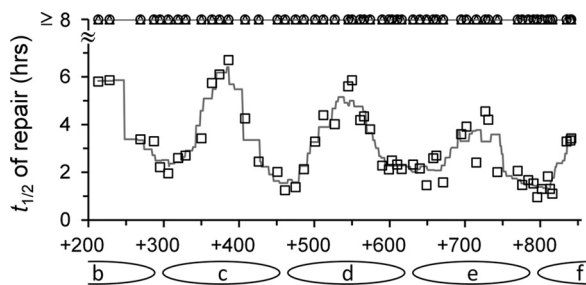
To determine the role of Dot1 in GGR, we measured repair of UV-induced CPDs in the NTS of the *RPB2* gene in wild-type and *dot1Δ* cells. A nucleotide resolution method that uses streptavidin magnetic beads and biotinylated oligonucleotides to facilitate isolation and strand-specific end-labeling of DNA fragments of interest was used for the measurement (17–19). The yeast cells were cultured to late log phase, UV-irradiated, and incubated in a repair medium for various lengths of time. Total genomic DNA was isolated, digested with a restriction enzyme to release the *RPB2* fragment, and incised at the UV-induced CPDs with an excess amount of T4 endonuclease V (20). The NTS of the restricted *RPB2* gene fragment was fished out, radioactively labeled at the 3′ end, and resolved on a DNA sequencing gel. The band intensities in the gel lane of 0 time repair indicate the yields of CPDs at these sites, and a decrease in band intensities at respective sites indicates repair of the damage (Fig. 1).

In wild-type cells, CPDs were repaired at different rates at different sites in the NTS of the *RPB2* gene (Figs. 1A and 2). The repair rates correlated generally well with nucleosome positioning, being slowest in the central regions of nucleosomal core DNA and fastest in the internucleosomal linker regions (Figs. 1A and 2). This indicates that a nucleosome structure inhibits GGR, in agreement with previous reports (e.g. Refs. 23, 24). In *dot1Δ* cells, no obvious repair can be seen in the same sequence in the period of 4 h (Figs. 1B and 2), indicating that Dot1 plays an important role in GGR throughout the NTS, including the internucleosomal linker regions. A longer time of repair incubation was not carried out because (i) most NER events in the yeast occur in the initial hours of repair incubation, and (ii) significant cell growth may occur after 4 h of repair incubation, which may obscure the fraction of repaired DNA in the samples.

***Methylation of H3K79 Is Also Required for GGR***—Dot1 has been shown to be required for methylation of H3K79 (12). To determine whether the role of Dot1 in GGR is accomplished by methylating H3K79, we measured GGR in cells whose genomic histone H3 genes (*HHT1* and *HHT2*) were deleted and complemented with a plasmid encoding the K79A mutant histone H3

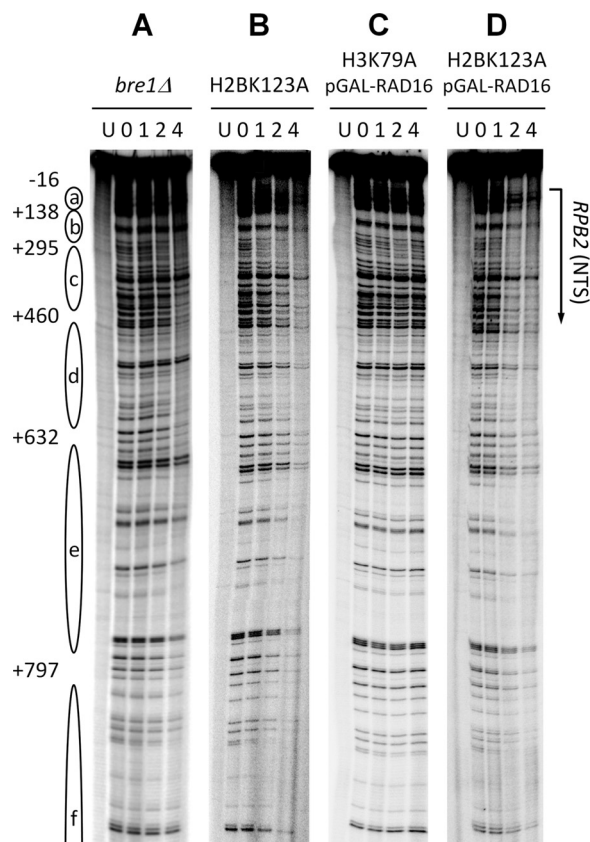


**FIGURE 1. Gels showing repair of CPDs in the NTS of the *RPB2* gene in wild type (WT), *dot1Δ*, and H3K79A cells.** The lanes are DNA samples from unirradiated (U) and UV-irradiated cells following different times (hours) of repair incubation. Ovals on the left represent positioned nucleosomes. Numbers on the left indicate nucleotide positions (relative to the transcription start site) at the centers of nucleosome linker regions, which is based on the systematic reference map of nucleosome positions across the yeast genome (40). The arrow on the right indicates the transcription start site.



**FIGURE 2. Plot showing repair of CPDs in the NTS of the *RPB2* gene in wild type, *dot1Δ*, and H3K79A cells.** The times (hours) required for repairing 50% ( $t_{1/2}$ ) of CPDs at individual sites along the NTS of the *RPB2* gene in wild-type (squares), *dot1Δ* (triangles), and H3K79A (circles) cells are plotted. The  $t_{1/2}$  values  $>4$  h were obtained by extrapolation of regression of the data obtained from 0, 1, 2, and 4 h of repair. The gray lines are smoothed  $t_{1/2}$  values, which were carried out by averaging the individual  $t_{1/2}$  values at continuous intervals of 40 nucleotides where the 40-nucleotide brackets were ramped along the DNA by 1 nucleotide. Ovals at the bottom represent nucleosome positions along the *RPB2* gene region analyzed. Nucleotide positions are numbered from the transcription start site of the gene.

(H3K79A) (15). Like *dot1Δ* cells, the H3K79A mutant cells showed no repair in any sites of the NTS of the *RPB2* gene (Figs. 1C and 2), indicating that Dot1 may mediate GGR by methylating H3K79.



**FIGURE 3. Gels showing repair of CPDs in the NTS of the *RPB2* gene in *bre1Δ* and H2BK123A cells, and in H3K79A and H2BK123A cells transformed with a plasmid overexpressing Rad16 (pGAL-RAD16).** Labels are the same as those shown in Fig. 1.

*Bre1 and Histone H2B Lysine 123 (H2BK123) Ubiquitination Are Partially Required for GGR*—Dot1 catalyzes mono-, di-, and trimethylation of H3K79 (12). Monoubiquitination of H2BK123, which is catalyzed by the ubiquitin E3 ligase Bre1, has been shown to be partially required for dimethylation and absolutely required for trimethylation but is dispensable for monomethylation of H3K79 (25, 26). To determine whether the methylation states of H3K79 affect GGR, we analyzed *bre1Δ* cells and those whose genomic histone H2B genes (*HTB1* and *HTB2*) were deleted and complemented with a plasmid encoding the K123A mutant histone H2B (H2BK123A) (15). As can be seen in Figs. 3, A and B, and 4, GGR was still apparent but significantly compromised in these mutant cells. These results indicate that (i) trimethylation of H3K79 may contribute to but is not absolutely required for GGR, and (ii) lower levels of methylation (mono- and dimethylation) at the lysine 79 also promote GGR.

*Overexpression of Rad16 Does Not Restore GGR in Cells Expressing H3K79A or H2BK123A Mutant Histones*—Loss of Dot1 or H3K79 methylation has been shown to have no or only a very minor effect on genome-wide transcription levels (27). Also, histone H3 K4R and K79R mutations (H3K4R/K79R), which prevent methylation at both lysine 4 and 79, do not affect expression of all NER genes tested (28). However, there was a 50% decrease in the *RAD16* mRNA in the H3K4R/K79R mutant cells after UV irradiation compared with  $\sim 2$ -fold increase in wild-type cells (28). To address the possibility that the deficient



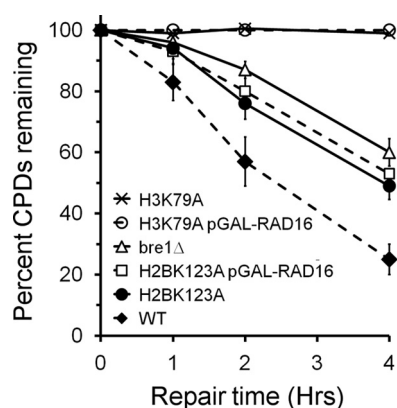


FIGURE 4. Plot showing repair of CPDs in the NTS of the *RPB2* gene in wild-type (WT), *bre1Δ*, H3K79A, and H2BK123A cells, and in H3K79A and H2BK123A cells transformed with a plasmid overexpressing Rad16 (pGAL-RAD16). The values shown are means ( $\pm$  S.E. (error bars)) of percent CPDs remaining at individual sites in the NTS of the *RPB2* gene at different repair times.

GGR we observed was due to lower levels of Rad16, we transformed H3K79A and H2BK123A cells with a plasmid bearing the *RAD16* gene (pGAL-RAD16) tagged with 3 $\times$ myc under the control of the *GAL1* promoter. Upon galactose induction, the Rad16 protein was overexpressed more than 10-fold. However, the overexpression did not affect GGR (Figs. 3 and 4), indicating that the effects of H3K79 methylation and H2BK123 ubiquitination on GGR are not caused by lower levels of Rad16.

**Dot1 and H3K79 Methylation Do Not Play Significant Roles in TCR**—To determine whether Dot1 and H3K79 methylation also play roles in TCR, we measured repair of CPDs in the TS of the *RPB2* gene in *rad16Δ* cells lacking Dot1 or expressing the H3K79A mutant. The reason for using *rad16Δ* cells is that these cells are deficient in GGR (4), so that TCR can be unambiguously determined. Following restriction digestion to release the *RPB2* fragment and incision at the CPDs with T4 endonuclease V, the TS of the *RPB2* gene fragment was fished out, radioactively labeled at the 3' end, and resolved on a DNA-sequencing gel. As can be seen in Figs. 5 and 6, rapid TCR, which initiates  $\sim$ 40 nucleotides upstream of the transcription start site of the *RPB2* gene, occurred in *rad16Δ*, *rad16Δ dot1Δ* and *rad16Δ* H3K79A cells, indicating that Dot1 and H3K79 methylation do not play a significant role in TCR. In agreement with previous reports (e.g. Refs 23, 24), TCR in these cells was not significantly modulated by nucleosome positioning (Fig. 5).

## DISCUSSION

In this paper, we present evidence that Dot1 and H3K79 methylation are required for GGR but dispensable for TCR. Dot1 and H3K79 methylation have been shown to be required for important aspects of DNA damage checkpoint activation (14). The roles of Dot1 and H3K79 methylation in GGR are unlikely to be achieved indirectly by activating the DNA damage checkpoint. First, *dot1Δ* strains largely share the checkpoint defects of *bre1Δ* strains, implying that the checkpoint role of Bre1 (through monoubiquitination of H2BK123) is mostly manifested through its ability to permit di- and trimethylation of H3K79, although deleting *SET1* (and thus blocking histone H3K4 methylation) as well as *DOT1* is required to replicate the

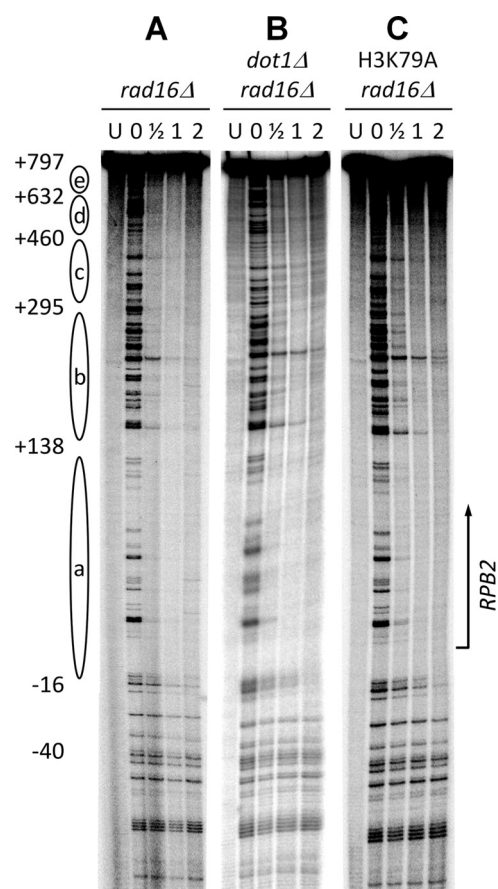


FIGURE 5. Gels showing repair of CPDs in the TS of the *RPB2* gene in *rad16Δ*, *rad16Δ dot1Δ*, and *rad16Δ* H3K79A cells. The lanes are DNA samples from unirradiated (U) and UV-irradiated cells following different times (hours) of repair incubation. Ovals on the left represent positioned nucleosomes. Numbers on the left indicate nucleotide positions (relative to the transcription start site) at the centers of nucleosome linker regions, which is based on the systematic reference map of nucleosome positions across the yeast genome (40). The arrow on the right indicates the transcription start site.

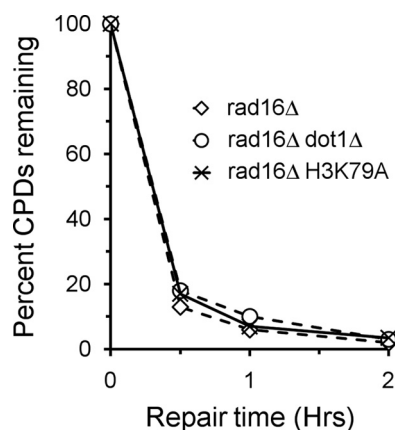


FIGURE 6. Plot showing repair of CPDs in the TS of the *RPB2* gene in *rad16Δ*, *rad16Δ dot1Δ*, and *rad16Δ* H3K79A cells. The values shown are means of percent CPDs remaining at individual sites in the coding region of the TS of the *RPB2* gene at different repair times. The standard error bars are within the symbols.

full checkpoint defect of *bre1Δ* strains (14). However, although compromised, GGR is still apparent in *bre1Δ* and H2BK123A mutant cells, indicating that di- and trimethylation of H3K79 contributes to but is not absolutely required for GGR. Second,

cells lacking *MEC1*, which plays the most important role in the checkpoint activation in the yeast (29), have little defect in GGR (data not shown). However, introduction of mutations to *mec1Δ* cells that disrupt H2BK123 ubiquitination or H3K79 methylation significantly decrease or abolish GGR, respectively (not shown), indicating that the histone modifications play much more important roles in GGR than the checkpoint activation.

Although chromatin structures can restrict the NER machinery from accessing sites of DNA damage, limited pieces of evidence have emerged recently that chromatin metabolism may also play an active role in the repair process (9). For example, acetylation of histone H3K9 and/or H3K14 by the acetyltransferase Gcn5 facilitates GGR (30, 31). Also, SWI/SNF, an ATP-dependent chromatin-remodeling complex, has been shown to be recruited to UV-damaged chromatin DNA (32). However, the effects of chromatin modifications/remodeling on NER in living cells documented so far are generally quite modest. In sharp contrast, H3K79 methylation by Dot1 appears to play a pivotal role in GGR. Lysine 79 of the two histone H3 molecules contained in a nucleosome are located at the top and bottom surfaces of the nucleosome disc and most likely regulate interactions with exogenous proteins (33). The lysine 79 with methyl moieties may serve as a docking site for the GGR machinery on the chromatin. In the absence of the methyl moieties, the GGR machinery may be excluded from the chromatin, including the vicinities of internucleosomal linker regions. Indeed, all GGR-specific factors identified so far, including Rad7, Rad16 (22, 34), Elc1 (3), and Dot1, are required not only for repair in nucleosome core regions but also in internucleosomal linker DNA.

Lesion processing by NER factors has been shown to be required for activation of the checkpoints in response to UV radiation (35). It is therefore reasonable to suggest that the roles of Dot1 and H3K79 methylation in the DNA damage checkpoint activation (14) may be indirectly achieved by their mediation of GGR. This explanation agrees with the observation that Dot1 and H3K79 methylation are epistatic to *RAD1*, which is essential for NER (both GGR and TCR) (13).

Approximately 90% of all histone H3 are methylated on K79 in the yeast (12, 36). H3K79 methylation is ~10-fold lower (but still 8–10-fold higher than background) at all Sir-dependent silenced regions, such as the telomeric and silent mating-type loci, but not at other transcriptionally repressed regions, such as the *TSL1* gene and the promoters of the repressed *SUC2* and *INO1* genes (36). Indeed, most genes have nucleosomes modified at H3K79; there was little correlation between the relative levels of H3K79 methylation at genes and transcriptional activity (36, 37). The widespread feature of H3K79 methylation makes it ideal for mediating GGR. Telomeres (38) and centromeres (39) are refractory to NER, which may be partly due to hypomethylation of H3K79.

A previous study showed that yeast cells expressing K79R mutant histone H3 have impaired NER at the transcriptionally silent mating-type locus *HML*, while maintaining nearly normal NER in the constitutively expressed *RPB2* gene and transcriptionally repressed *GAL10* gene (28). This study collectively measured NER in both strands of the different loci (*i.e.* did not distinguish the two strands), which may have missed the detec-

tion of repair defect in the NTS of the *RPB2* gene. There is evidence that GGR and TCR compete for common NER factors; specific elimination of GGR may enhance the rate of TCR in the cell (22). The observation that H3K79 methylation does not affect overall NER in the repressed *GAL10* gene agrees with our results, which indicate that a defect in GGR does not cause a significant slowdown of overall NER in the repressed *GAL1–10* genes, presumably due to enhanced TCR mediated by noise transcription at the repressed loci (Ref. 22 and data not shown).

In summary, we identified a novel GGR-specific NER factor (Dot1) and unveiled a critical link between a histone modification (H3K79 methylation) and the GGR process. These findings may open up new avenues of research regarding the fascinating mechanisms of how chromatin is actively engaged in NER.

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